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LETTER TO THE EDITOR

Male Health

Clinical features and an atypical *WT1* mutant site in a child with incomplete Denys–Drash syndrome

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Dear Editor,

Denys–Drash syndrome (DDS) is characterized by the triad of progressive nephropathy, urogenital malformation and Wilms' tumor. Incomplete variants present with nephropathy with either intersex disorders or Wilms' tumor.[1] Most DDS patients carry *WT1* mutations in exon 8 or 9.[1] Here, we report an incomplete DDS child who carried a novel *WT1* missense mutation in exon 6 and had unique clinical manifestations.

The subjected child' age was 11 years and 9 months. His father is healthy and his mother died suddenly after giving birth to his younger brother. The patient was born at full-term birth without asphyxia. He was found to have abnormal external genitalia after birth and had been raised as a girl. He learned to speak between 2- and 3-years-old and learned to walk between 4- and 5-years-old. He also had difficulties in learning at school. The child was not sent to hospital until his penile development was noticed at 10 years and 11 months. Ultrasound revealed microcalcifications in the left testis, right cryptorchidism and a right testis in the right iliac fossa. Magnetic resonance imaging revealed that prostate and seminal vesicles dysplasia could not be ruled out. Karyotype was 46,XY. Right testicular descent surgery was performed. His urinary protein was observed positive (+ – ++) 5 months later. He was admitted to our department for the unexplained proteinuria at 11 years and 9 months. Regular rhythm and a precordial grade II/VI systolic murmur were heard. The penis was developing and scrotum was symmetrical dehisced. The urethral opening was located in the rift, with little pubes on the symphysis pubica. The volume of the left testis was approximately 6 ml, and the volume of the right one was approximately 0.5 ml. Ultrasound revealed kidneys of normal size and echoes. Echocardiography revealed a persistent left superior vena cava and mitral valve prolapse with mild regurgitation as well as an enlargement of the left atrium, left ventricle and right ventricle (diameter of right atrium 31 mm, right ventricle 18 mm, left atrium 29 mm, left ventricular 39 mm and ejection fraction of left ventricular was 68%). Urinary protein was 10.6 mg kg^{-1} d^{-1}, serum creatinine 50 μmol l^{-1} and albumin 41.8 g l^{-1}. The hormones test revealed follicle-stimulating hormone 22.25 IU l^{-1} (1–8 IU l^{-1}); luteinizing hormone 12.34 IU l^{-1} (2–12 IU l^{-1}); and prolactin, estradiol, testosterone and progesterone were all in normal range. Renal histopathology revealed mild segmental hyperplasia in mesangial cells and matrix and minimal change disease was indicated (**Figure 1a** and **1b**). The child has not received specific therapy except for fosinopril. Urinary protein is still positive (+ – ++) at present and renal function is within normal range.

WT1 mutation in exon 6, c.893A>T (p.Q298L) was identified in the patient (**Figure 1c** and **1d**), which was not found in his father (**Figure 1e**). This mutation is absent from 100 control chromosomes.

Alignment of the WT1 protein across six different species revealed that glutamine is conserved at the 298th position in human, chimpanzee, dog, rat, mouse and zebrafish (**Figure 1f**). The missense mutation p.Q298L is predicted to cause the loss of an α helix in vicinity of the mutant position (**Figure 1g** and **1h**).

WT1 is located at human chromosome 11p13 and contains 10 exons.[2] Exons 1–6 of *WT1* encode an amino-terminus rich in proline and glutamine, which regulates gene expression. Exons 7–10 encode four contiguous Cys2-His2 zinc finger regions, which function as DNA- or RNA-binding domains.[2] Mutant WT1 can lead to glomerular maturation disorders and genitourinary malformations. Mutations in exon 8 and 9 of *WT1* have been reported the most. There have been only a few reports of truncate mutations in exons 1–6 that cause DDS.[3-5] Missense mutation c.903A > G (p.Q298L) in exon 6, which was detected in the present patient, is a novel mutation. This mutation causes a substitution of leucine for glutamine which leads to a helix lost in the peptide chain.

DDS patients usually present with congenital or early onset proteinuria and progress to end-stage renal disease before 4 years.[1] The most common histology of DDS is diffuse mesangial sclerosis; whereas, minimal change disease can also be observed occasionally.[6] In the present case, proteinuria was discovered at 11 years and 4 months, and renal histopathology was minimal change disease. It has been reported that *WT1* nephropathy caused by *WT1* mutations in exons 1–6 had a mild phenotype.[3-5] Although renal function is currently normal in this child; proteinuria is persistent with the administration of angiotensin converting enzyme inhibitor (fosinopril). Because *WT1* nephropathy is steroid resistant, calcineurin inhibitors (cyclosporine A or tacrolimus) is on consideration for next treatment in this child based on the benefits observed in literature[7] and our previous experience.[1,8]

Urogenital abnormalities, including male pseudohermaphroditism, cryptorchidism, hypospadias and penile hypoplasia, mainly occur in DDS patients with 46,XY karyotype.[5] Urogenital malformations are prominent in the present case, though his nephropathy is mild.

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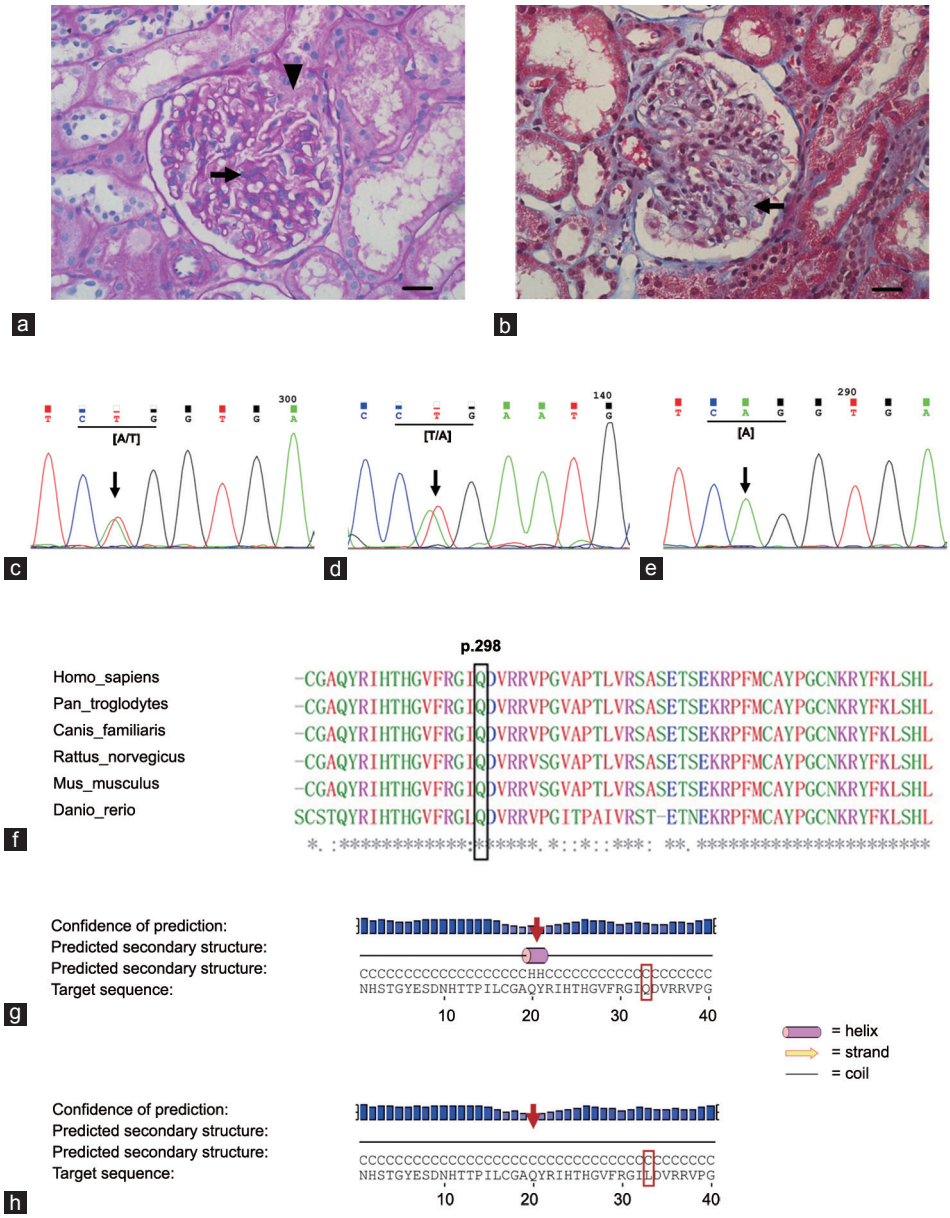

Figure 1: Renal histopathology, sequencing results of *WT1* and bioinformatic analysis of mutant *WT1*. (**a**) Periodic acid-Schiff staining and (**b**) Masson trichrome staining (light microscope, bars: 30 μm both in **a** and **b**). Renal pathology of the boy revealed mild lesion of glomeruli, urinary protein leakage in the Bowman's capsule (arrowhead in **a**) and mild segmental proliferation of mesangial cells (arrow in **a**) and mild segmental accumulation of matrix (arrow in **b**). (**c** and **d**) Forward (**c**) and reverse (**d**) sequencing results of the patient show the change at position c.893 A > T. The arrows indicate the position of the mutation. The underlining denotes the codon that contains the mutation. (**e**) The sequencing results of the patient's father; no mutation was detected. (**f**) Alignments of amino acid sequences. The glutamine at position p.298 was conserved in the amino acid sequences across the species examined (asterisks present 100% conserved regions, colons presents conservative substitution regions and black spots present nonconservative substitution regions). (**g** and **h**) Predicted structures of wild (**g**) and mutant (**h**) WT1 proteins across species are displayed by the Inter pro (http://www.ebi.ac.uk/interpro/) and PROSITE databases (http://www.expasy.ch/prosite/). Secondary structure predictions for exon 6 of the wild-type protein (**g**) and the mutant (c.893 A > T (p.Gln298Leu)) protein in the patient (**h**) were generated using the PSIPRED Protein Structure Prediction Server (http://bioinf. cs.ucl.ac.uk/psipred/). The mutant *WT1* protein lost an α helix near the mutant site (arrow).

Gonadotropin disorders have been reported in a few patients with *WT1* mutations.[9] Most of these patients had genitourinary malformations and significantly elevated luteinizing hormone and follicle-stimulating hormone, but normal range testosterone similar to the present case. These disorders may indicate that the child's gonads had a low response to gonadotropins, as seminal vesicles dysplasia has not been ruled out in this child by magnetic resonance imaging, abnormal gonadal development should be considered.

Wilms' tumor has been observed in all reported *WT1* neprohopathy cases with *WT1* mutations in exons 1–6.[3–5] Thus, the present boy is at high risk of developing Wilms' tumor and should receive periodic medical examination.

Cardiovascular abnormalities have been observed in the present case. It was reported that *WT1* is expressed in the epicardium and subepicardial mesenchymal cells and is required for normal vascularization in heart development.[10] There has been no report

on mental retardation in DDS patients to date. Whether the heart abnormalities and mental retardation are attributed to the detected *WT1* mutation deserves more study.

In summary, this study reports an incomplete DDS boy with unique manifestations and an atypical *WT1* mutant site. This study may help to clarify the genotype to phenotype relations of DDS patients.

AUTHOR CONTRIBUTIONS

HYW and ZHY carried out the experiments and clinical data collection and drafted the manuscript. JCM, YM and JJS helped collecting some clinical data and the blood samples. LZS conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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